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Avaliações físico-químicas, microbiológicas e bioativas de polpa de Araçá-Boi (*Eugenia stipitata* Mc Vaugh) exposta à Irradiação Gama

Physicochemical, microbiological and bioactive evaluations of 'Araçá-Boi' (*Eugenia stipitata* Mc Vaugh) pulp exposed to Gamma Irradiation

Evaluaciones fisicoquímicas, microbiológicas y bioactivas de la pulpa 'Araçá-Boi' (*Eugenia stipitata* Mc Vaugh) expuesta a la Radiación Gama

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Resumo

Objetivou-se neste trabalho avaliar a qualidade físico-química, microbiológica e bioativa de polpas de Araçá-Boi expostas à Irradiação Gama. A colheita manual foi realizada pela manhã, utilizando boas práticas agrícolas, e as frutas foram acondicionadas em caixas térmicas e transportadas para o Laboratório de Armazenamento e Processamento de Produtos Agrícolas da Universidade Federal de Campina Grande (LAPPA/UFCG), para obtenção da polpa. A polpa de ‘Araçá-Boi’ foi irradiada no Laboratório de Irradiação Gama do Centro de Desenvolvimento de Tecnologia Nuclear (CDTN), localizado na UFPE - Recife, PE, onde três doses de Irradiação Gama (2, 3, 4 kGy) foram aplicadas para comparação posterior com a amostra não irradiada (controle). Após a irradiação, foram avaliados os parâmetros microbiológicos, físico-químicos e a quantificação de vitamina C e flavonóides, observando-se que a dose 6 é agrupada em um conjunto com características diferenciais dos demais tratamentos, pois proporcionou maior AA, pH, luminosidade e atividade de água. Observou-se que não houve desenvolvimento de microrganismos após irradiação em todos os tratamentos analisados. Conclui-se que as diferentes doses de Irradiação Gama garantiram a qualidade microbiológica da polpa de Araçá-Boi, permanecendo aptas ao consumo e em conformidade com a legislação brasileira.

Palavras-chave: Processamento não térmico; Compostos bioativos; Vitamina C; Validade.

Abstract

The aim of this research was to evaluate the physicochemical, microbiological and bioactive quality of ‘Araçá-Boi’ pulps exposed to Gamma Irradiation. Manual harvesting was done in the morning, using good agricultural practices, and fruits were packed in thermal boxes and transported to the ‘Laboratório de Armazenamento e Processamento de Produtos Agrícolas’ of the 'Universidade Federal de Campina Grande', to obtain the pulp. The 'Araçá-Boi' pulp was irradiated in the 'Laboratório de Irradiação Gama do Centro de Desenvolvimento de Tecnologia Nuclear' (CDTN), located at UFPE - Recife, PE, where three doses of Gamma Irradiation (2, 3, 4 kGy) were applied for later comparison with the non-irradiated sample (control). After irradiation, the microbiological, physicochemical parameters and the quantification of vitamin C and flavonoids were evaluated it can be observed that dose 6 is grouped in a set with differential characteristics of the other treatments, as it provided higher AA, pH, luminosity and water activity (A_w). It was observed that there was no microorganism development after irradiation in all analyzed treatments. It can be concluded that the different doses of Gamma Irradiation guaranteed the microbiological quality of the 'Araçá-Boi' pulp, remaining fit for consumption and in compliance with the Brazilian legislation.

Keywords: Non-thermal processing; Bioactive compounds; Vitamin C; Shelf life.

Resumen

El objetivo de este trabajo fue evaluar la calidad físico-química, microbiológica y bioactiva de las pulpas de 'Araçá-Boi' expuestas a la Irradiación Gama. La recolección manual se realizó por la mañana, utilizando buenas prácticas agrícolas, y las frutas se empacaron en cajas térmicas y se transportaron al 'Laboratório de Armazenamento e Processamento de Produtos Agrícolas' da 'Universidade Federal de Campina Grande', para obtener la pulpa. La pulpa de 'Araçá-Boi' se irradió en el 'Laboratório de Irradiação Gama do Centro de Desenvolvimento de Tecnologia Nuclear' (CDTN), ubicado en UFPE - Recife-PE, donde se aplicaron tres dosis de Irradiación Gama (2, 3, 4 kGy) para una comparación posterior con la muestra no irradiada (control). Después de irradiación, se evaluaron los parámetros microbiológicos, físico-químicos y la cuantificación de la vitamina C y los flavonoides, observando que la dosis 6 se agrupa en un conjunto con características diferenciales de los otros tratamientos, ya que proporciona mayor AA, pH, luminosidad y actividad de agua (A_w). Se observó que no hubo desarrollo de microorganismos después de la irradiación en todos los tratamientos analizados. Se concluye que las diferentes dosis de irradiación gamma garantizaron la calidad microbiológica de la pulpa 'Araçá-Boi', siendo aptas para el consumo y cumpliendo con la legislación brasileña.

Palabras clave: Procesamiento no térmico; Compuestos bioactivos; Vitamina C; Duracion.

1. Introduction

The 'Araçá-Boi' (*Eugenia stipitata* Mc Vaugh) is a fruit tree of the *Mirtacea* family from the Peruvian Amazon, usually cultivated in Brazil, Peru and Bolivia. In Brazil it is part of native species and cultivated in the Amazon biome with much flavor characteristic appreciated by the local population (Sacramento, 2008).

The perishability of this fruit is seen as a reason for making its consumption unfeasible in different regions other than those of its natural occurrence (Viana et al., 2012). Thus, the use of conservation technologies is fundamental in maintaining their functional and nutritional properties, enabling the reach of new markets and thus there "in natura" use for other purposes without the need for processing (Sanches et al., 2017).

The 'Araçá-Boi' fruit has been evaluated for its nutrient content and utilization in food products, such as juices, jellies and sweets, is a fruit rich in fiber and vitamin A, B and C (Neri-Numa et al., 2013). It has nutritional and functional potential, demonstrating good antioxidant activity and high phenolic content, as well as high protein and carbohydrate rates.

Fruits taste similar to guava, although slightly more acidic and more fragrant, they can be consumed as an ingredient in the production of sweets, ice cream and beverages (Franzon et al., 2009). The fruit pulp agroindustry has aroused interest in the study of different ways of preserving food due to the growing consumer demand for natural products that have a long shelf life, coupled with the growing demand for tropical fruits domestically and abroad. In this context, the pulp of the fruit of 'Araçá-Boi' can be framed.

However, one of several problems involving the marketing of fruit pulp is its high perishing, as well as the costs in the cold chain, making it difficult to transport and store in centers far from the collection and processing site.

This scenario has contributed to the development of emerging technologies, processes that do not use classic heat treatment for food preservation, or that eliminate the use of the cold chain during storage, which may lead to the availability of products with better sensory, nutritional and prolonged shelf life.

Gamma Irradiation is a non-thermal conservation method that provides durability many times longer than pasteurization, it does not influence the appearance and composition of the food (Farkas, 2006). The Food Agriculture Organization (FAO), World Health Organization (WHO) and the International Atomic Energy Agency (IAEA) concluded in a report prepared in 1981 that irradiation of food up to a dosage of 10 Kg does not result in toxicological and non-toxic damage offers risks (Farkas & Mohácsi-Farkas, 2011).

More than 50 countries have already approved around 60 foodstuffs to be irradiated for local consumption and, or for export, and approximately 40 countries are using food irradiation (Kume et al., 2009). Ionizing radiation is a proven safe process and has been evaluated in many applications.

Due to the dose applied to the food, there is an improvement in the microbiological quality of the product, resulting in reduced storage losses and longer shelf life (Khan & Abrahem, 2010). Therefore, the aim of this study was to evaluate the physicochemical, microbiological and bioactive quality of 'Araçá-Boi' pulp exposed to Gamma Irradiation.

2. Material and Methods

2.1. Research methodology

The research was carried in laboratory and the methodology used was quali-quantitative (scientific statistical method) (Pereira et al., 2018). This study was based on research studies of Farkas (2006), IAL (2005), AOAC (2005), APHA (2001), Rodriguez-Amaya & Kimura (2004), Benassi & Antunes (1988), Hunter, Richard & Harold (1987), Francis (1982), Somogyi (1945) and Nelson (1944).

2.1 Obtaining fruit samples

Fruits of 'Araçá-Boi' were purchased from Amizade Vila Brasil Farm, Uma city, Bahia State from Brazil. Used fruits were selected according to the ripening stage, where they were used, those that fit within the established standards for commercialization.

The manual harvesting was done in the morning, using good agricultural practices. The fruits were packed in thermal boxes and transported to the 'Laboratório de Armazenamento e Processamento de Produtos Agrícolas' of the "Universidade Federal de Campina Grande' (LAPPA/UFCG), Campina Grande city, Paraíba State from Brazil.

2.2 Processing fruit samples

The fruits were selected, washed and pulped using a horizontal pulping machine with a 0.8 mm diameter sieve. They were then packed in polyethylene plastic bags containing 100 g

/ unit and frozen (-18 ºC) until the application of the treatments. This process was done from the Food Processing and Drying Laboratory of the UFCG.

2.3 Application of Gamma Irradiation

The pulps were transported in under refrigeration to the Gamma Irradiation Laboratory of the Center for the Development of Nuclear Technology (CDTN) in Federal University of Pernambuco (UFPE), Recife city, Pernambuco State from Brazil. The laboratory has a cobalt 60 radiator chamber that maintains an international safety standard for radiator operation. Samples were divided and received different doses of Gamma Irradiation (2, 3, 4 kGy) for later comparison with the non-irradiated sample (control). After the Gamma Irradiation process, the pulp was stored at 4 ºC for 60 days to be characterized.

2.4 Evaluation of physical parameters

The color of the irradiated pulp was determined by HunterLab colorimeter (Hunter, Richard & Harold, 1987). The color values were expressed according to the CIELAB coordinate system, where the variables L * (brightness), a * (red-green component) and b * (yellow-blue component) were used to calculate the color tone (ho) and color saturation (C *)

2.5 Evaluation of physicochemical parameters

The following parameters were analyzed: soluble solids content (SS in ºBrix), pH, total titratable acidity (ATT) and SS/ATT ratio (ratio) according to IAL (2005). After cooling, the following physicochemical analyzes were performed on the product: soluble solids content, pH, total titratable acidity, ratio, reducing and total sugars, ash and humidity, according to IAL (2005).

Spectrophotometry determined the content of reducing and total sugars (Somogyi, 1945; Nelson, 1944), and the acid hydrolysis step was performed according to IAL (2005). Bioactive compounds analyzes were performed at the ‘Laboratório de Armazenamento e Processamento de Produtos Agrícolas’ of the ‘Universidade Federal de Campina Grande’ (LAPPA-UFCG). Determination of the ascorbic acid content of fruit pulps will be based on the oxidation of ascorbic acid by the 2,6-dichlorophenolindophenol reagent (Benassi &

Antunes, 1988; AOAC, 2005). Results were expressed as mg for ascorbic acid per 100 g of pulp.

The yellow flavonoids of the pulps will be evaluated according to the methodology described by Francis (1982) based on their extraction with ethanol (95%) and 1.5 M HCl (85:15, v: v). The absorbance reading will be performed in a spectrophotometer, with the yellow flavonoid content expressed in per 100 g of pulp as described by Silva et al. (2014).

Total carotenoid content was determined according to the method described by Rodriguez-Amaya & Kimura (2004). Absorbance reading was performed using a spectrophotometer. The total carotenoid content was calculated from the value of the β-carotene absorption coefficient in petroleum ether and expressed as μg β-carotene per 100 g pulp.

2.6 Evaluation of pulp microbiology

Microbial analysis was performed according to recommendations by the RDC Resolution Number 12, of January 2, 2001, and the samples were analyzed according to the methodology described by the American Public Health Association (APHA, 2001), to determine total coliforms, thermotolerant coliforms, viable aerobic mesophilic bacterium and Salmonella sp.

The analyzes of total and thermotolerant coliforms were performed following the technique of the most probable number. The analysis of viable aerobic mesophilic bacteria was performed by the deep plate technique using Plate Count Agar and incubation at 35 ± 2 °C for a period of 48 h.

For the analysis of Salmonella sp. the procedure was as follows: a pre-enrichment of the samples with lactose broth and incubation was carried out at 42 ± 2 °C for 24 h, and then a selective enrichment with Tetrionate and Selenite cysteine broth was done followed by incubation in xylose lysine deoxycholate Agar and Enteric Agar.

2.7 Statistical analysis

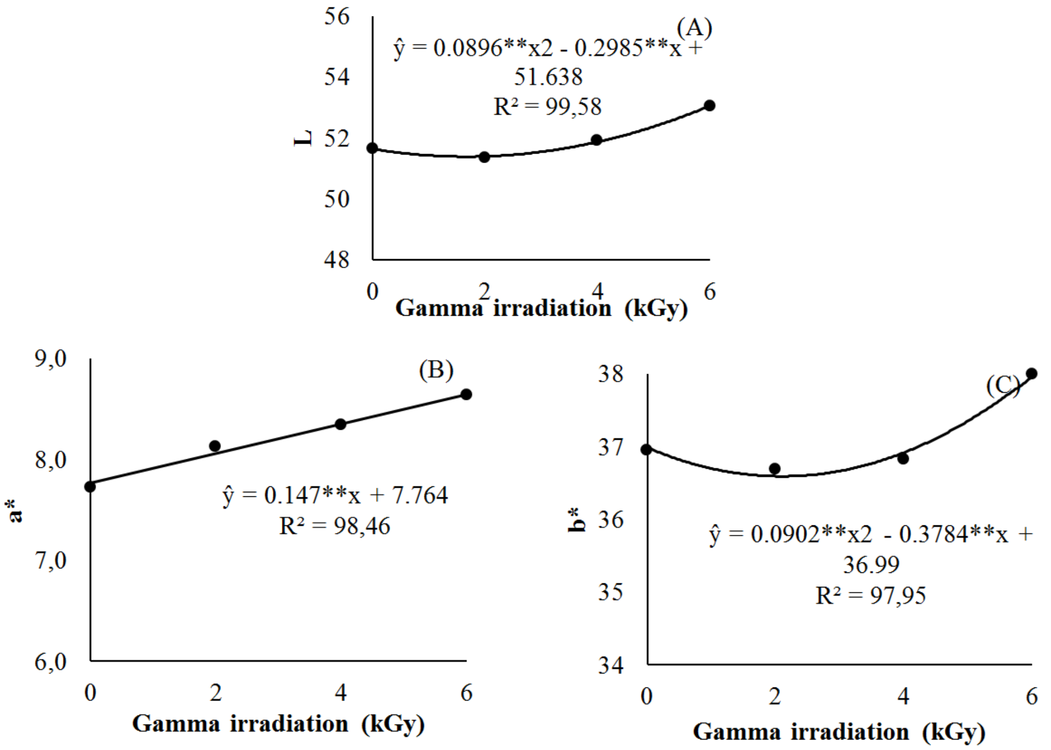
Data were subjected to analysis of variance (ANOVA) and based on the significance of the F test, polynomial regression analysis to test the effect of Gamma Irradiation doses, testing up to quadratic level, and significance of up to 5%, probability and coefficient of determination (R^2) above 60% the software SAS 9.3 (2011) was used. Hierarchical cluster analysis was also performed using the JMP® 10.0.0 DEMO software, using the Ward's method, to represent the similarity of radiation doses with respect to all fruit quality characteristics.

3. Results and Discussion

It was observed that luminosity (L*) values ranged from 51.40 to 53.14 with increasing irradiation doses, indicating a trend towards a lighter 'Araçá-Boi' pulp (Figure 1A). The average that increased the irradiation dose increased the coordinate values a * and b *, providing a gradual pulp darkening (Figure B and C).

The a* coordinate indicates the intensity from red to green (positive values are shades of red, and negative values, shades of green) and the b* coordinate indicates the intensity of yellow to blue (positive values are shades of yellow, and negative values, shades of blue). Positive values were found for the coordinates a* and b*, indicating a tendency for red and yellow tones.

Figure 1. Luminosity* (A), a* (B) and b* (C) pulp fruit colouring of 'Araçá-Boi' exposed to different doses of Gamma Irradiation.



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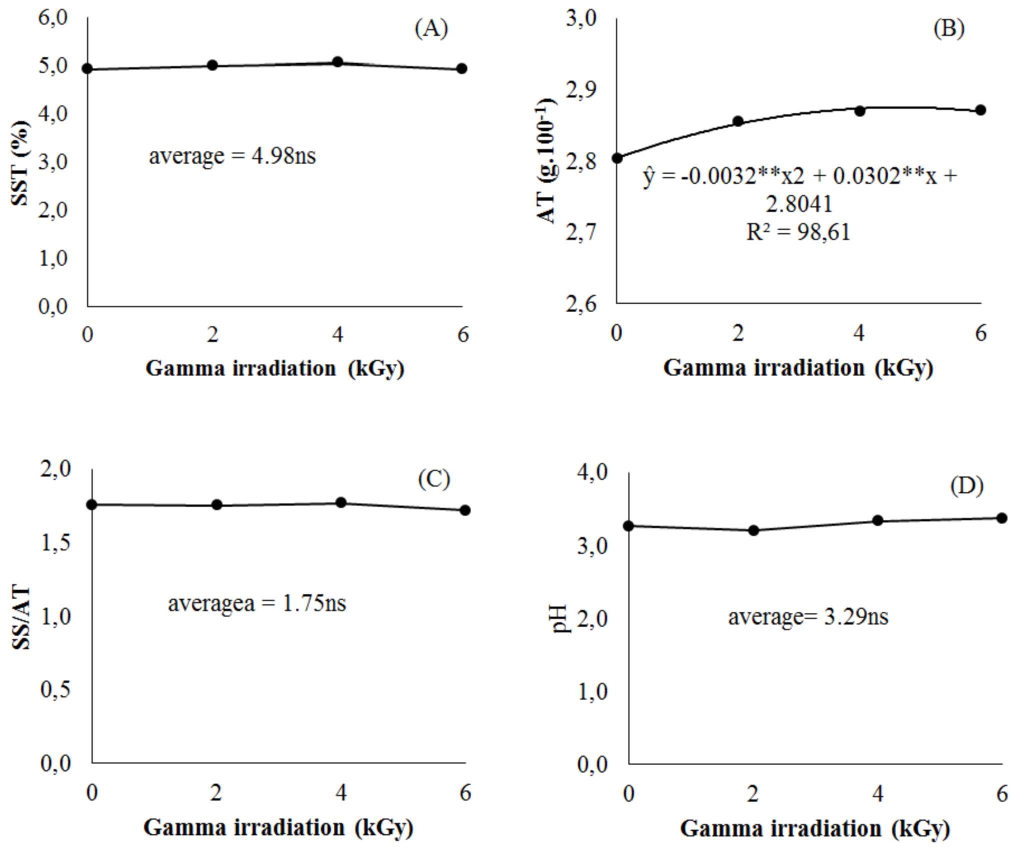
Soluble solids content practically did not change with the radiation application (Figure 2A), presenting an average value of 4.98 between the control sample and the doses tested, being close to the value found in Garzón et al. (2012) who reported for the soluble solids contents of 4.6 ° Brix. The data revealed that the applied Gamma Irradiation did not affect the soluble solids content of the 'Araçá-Boi' pulp, since the control (without irradiation) had a similar behavior to the others.

Figure 2. Total soluble solids (A), acidity titulation (B), SS/AT (C) relation and fruit (D) pH of 'Araçá-Boi' pulp exposed to different doses of Gamma Irradiation.



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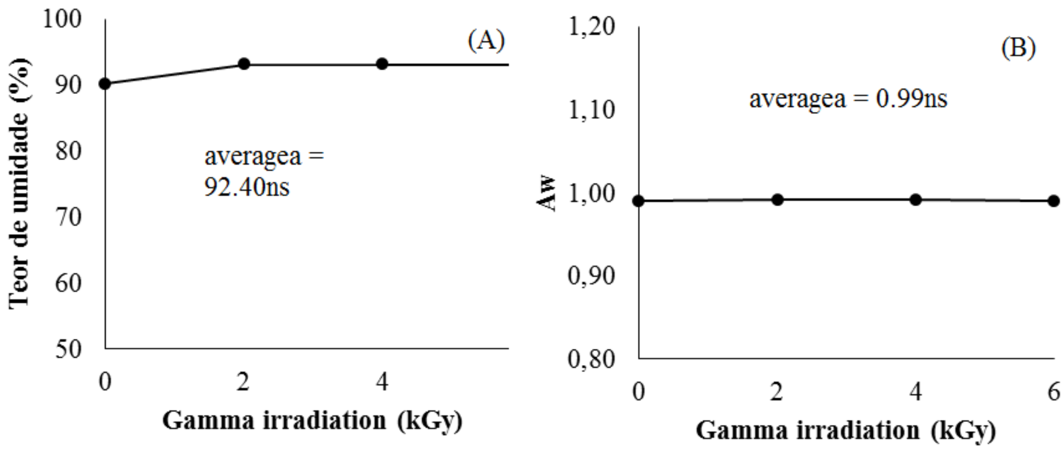
There was no significant difference (P <0.05) in acidity between the control and the irradiated samples (Figure 2B). However, there was an increase in acidity values with the application of treatments when compared to the control treatment. The acidity values ranged from 2.78 to 2.87. While Virgolin (2015), when evaluating the physicochemical characterization of the fruit pulps of abiu, 'Achachairu', 'Araçá-Boi', 'Biri-Biri' and 'Mangosteen' from the Amazon Biome, in different harvests, reported results for the 'Araçá-

Boi' fruit from 1.00 to 2.83 in the different harvests. According to Mota (2006), this difference in fruit acidity may be associated with the edaphoclimatic conditions of the crop.

Different irradiation doses did not significantly influence ($P > 0.05$) pH values in the 'Araçá-Boi' pulp (Figure 2D), being observed an average pH value of 3.29. The same happened in the work of Nassur (2016) applying doses of 0.0; 0.5; 1.0 and 1.5 KGy, aiming to extend the shelf life and quality maintenance of strawberries stored in refrigerated system (0 ± 1ºC and 90 ± 5% RH) for 15 days, where they obtained an average pH value of 3.5, and there was no significant difference ($P > 0.05$) between the irradiation dosages to affect the fruit pH during the cold storage. Silva (2013), in irradiated blackberry pulp with radiation doses of 0.75; 1.5 and 3 KGy, where pH values ranged from 2.89 to 2.99. Santillo (2011) where there was no significant difference for pH and soluble solids content (° brix) in grapes irradiated at 0, 0.5, 1, 1.5 and 2 KGy and stored for 21 days.

In all tested doses, it was observed that the values for water activity (A_w) were constant (Figure 3B), applied Gamma Irradiation did not interfere ($P > 0.05$) in the water activity (A_w) values of the 'Araçá-Boi' pulp, when the control (without irradiation) had similar behavior to the others.

Figure 3. Humidity means (A) and water activity (B) of 'Araça-Boi' pulp exposed to different doses of Gamma Irradiation.



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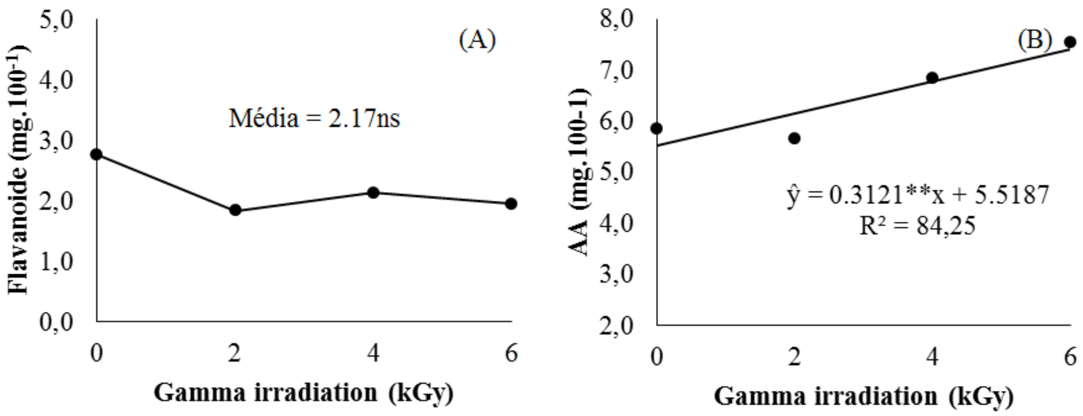
It was studied that there was no significant loss ($P > 0.05$) in the flavonoid values, however there was a decrease with the application of treatments when compared to the control treatment (Figure 4A). This may be due to the breakdown of the instability of some active compounds when exposed to visible and ultraviolet light or other sources of ionizing radiation

(Iacobucci & Sweeny, 1983). Similar results were found by Virgolin (2015) when evaluating the physicochemical characteristics for pulp of 'Abiu', 'Achachairu', 'Araçá-Boi', 'Biri-Biri' and yellow 'Mangosteen' fruits from different Amazonian harvests, reporting an average value of 2 , 21 for the results of flavonoids from the 'Araçá-Boi' fruits in 5 different seasons.

Different doses of irradiation interfered with the pulp ascorbic acid (AA) content (Figure 4B). It can be observed that with the increase of irradiation doses, they obtained higher AA retention results. This fact may be linked to the enzymatic deactivation provided by the increase of Gamma Irradiation doses. The AA values found for the 'Araçá-Boi' pulps were similar to those found by Virgolin (2015). They found average levels of 8.30 mg ascorbic acid per 100 g in acerola, 'Araçá' fruit from different harvests.

Figure 4. Yellow flavonoid (A) and ascorbic acid (B) of 'Araçá-Boi' pulp exposed to different doses of Gama Irradiation.



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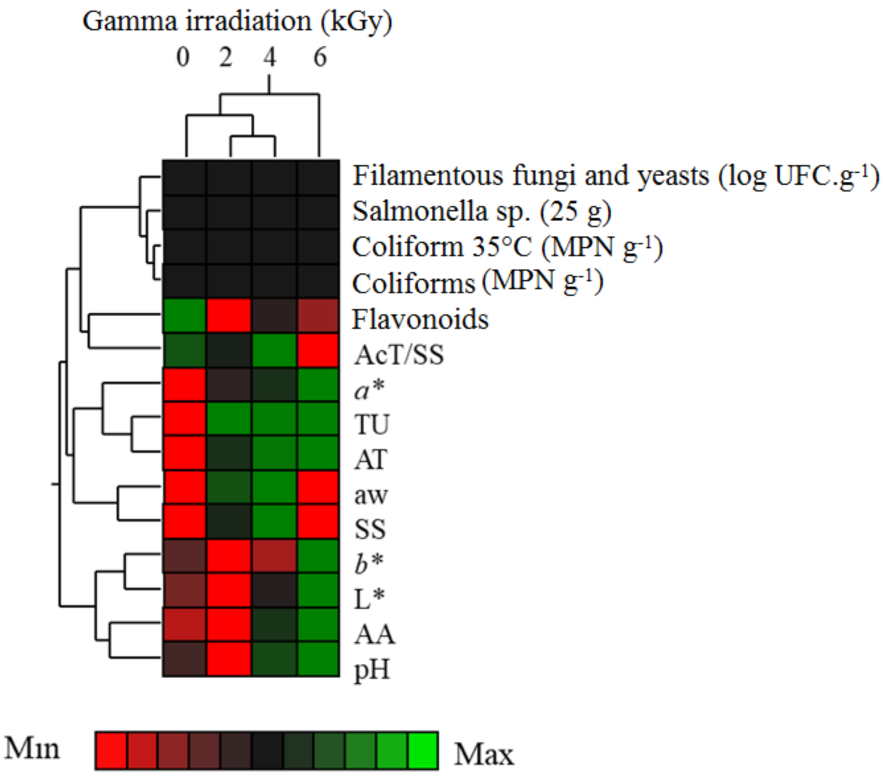
Based on the set of microbiological, physicochemical and bioactive analyzed characteristics, the dissimilarity dendrogram was elaborated, in which was observed the formation of distinct groups that showed some degree of dissimilarity (Figure 5). We can observe that dose 6 is grouped in a set with differential characteristics to other treatments, as it provided higher AA, pH, luminosity and water activity.

Figure 5. Non similarity square for different doses of Gamma Irradiation applied in 'Araçá-Boi' pulp, evaluated by cluster Ward's method through microbiological, physical, physical chemical and bioactive compounds.



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The Table 1 shows the results from microbiological parameters of 'Araçá-Boi' pulp after treatments with different doses of Gamma Irradiation. It was observed that there was no microorganism development after irradiation in all analyzed treatments (Table 1), being compatible with the standards required by Brazilian legislation, absence of *Salmonella* and Coliform count at 35 and 45 ºC always less than 3 (MPN), regardless of the dose applied.

Table 1. Microbiological parameters of 'Araçá-Boi' pulps after treatments with different doses of Gamma Irradiation.

Microorganisms	Gamma Irradiation (kGy)			
	0	2	4	6
Coliform 35 °C (MPN g^{-1})	<3	<3	<3	<3
Coliform 45 °C (MPN g^{-1})	<3	<3	<3	<3
Salmonella sp. (25 g)	Absent	Absent	Absent	Absent
Filamentous fungi and yeasts (log UFC g^{-1})	<100	<100	<100	<100

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In addition to the absence of coagulase positive *Staphylococcus* contamination, although RDC No. 12 of January 2, 2001 (ANVISA) does not set a limit value, the presence of these microorganisms could demonstrate deficiency in processing and handling at the time of preparation of the studied product.

Irradiation causes a variety of physical and biochemical effects on microorganisms. Once absorbed by a biological material, ionizing radiation can have direct or indirect action on the product that received this process (Fanaro, 2013).

In direct action, occurs the excitation or ionization of nucleic acid molecules and from this, biological changes that can lead to cell death will be conducted (Alcarde et al., 2003).

The sensitivity of macromolecules by radiation is approximately proportional to their molar mass, and a dose of 100 Gy would damage 2.8% of a bacterial cell's DNA, and this range could be lethal to a large fraction of living organisms, especially to living organisms complex.

These considerations explain why a given dose can have a lethal effect on microorganisms without changing (or slightly changing) the chemical composition of the irradiated food (Aquino, 2003).

These results are indicative that Gamma Irradiation may be effective to inhibit some microorganisms, as observed by Mostafavi et al. (2013), where it was determined that a 600 Gy dose was effective for complete inhibition of *Penicillium expansum*.

Tezotto-Uliana et al. (2013) evaluated in their work the efficiency of Gamma Irradiation associated with refrigerated storage in fresh raspberry. In this work, doses of 0.5 was used; 1.0 and 2.0 kGy, and the storage temperature was 0 °C.

It was possible to verify the synergistic effect of cold storage with the irradiation that allowed the raspberry to extend the shelf life for 8 days. Silva et al. (2014) evaluated the effects of Gamma Irradiation doses on quality conservation of blackberry pulp (*Rubus* spp. L.) concluded that 1.5 kGy doses ensured microbiological quality and increased shelf life 60 days of refrigerated storage.

4. Final Considerations

It can be concluded that the different doses of Gamma Irradiation guaranteed the microbiological quality of 'Araçá-Boi' pulp, remaining fit for consumption and in compliance with the legislation.

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Even though there is a reduction in flavonoid contents with the tested doses, use of irradiation treatment is feasible for this product, as they maintained the physicochemical and biotic compound characteristics in which the different doses of Gamma Irradiation did not change the pH, content of soluble solids, titratable acidity and vitamin C from 'Araçá-Boi' pulp.

The suggestions for future research is association of lower irradiation doses with other conservation methods can minimize the problems associated with sensory characteristics and, consequently, reduce the cost of the irradiation process.

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